

# From Diatom Biomolecules to Bioinspired Syntheses of Silica- and Titania-Based Materials

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## Abstract

Amorphous silica is (next to  $\text{CaCO}_3$ ) the second most abundant biologically produced inorganic material. A certain group of photosynthetic microalgae, called diatoms, forms complex 3D silica architectures (frustules) containing regularly arranged nanoscale features (pores, channels, protuberances). Recently, biomolecules involved in diatom silica formation have been characterized, and first insights into their structure-function correlations have been obtained. This has spurred the development of synthetic (bio)polymers capable of directing the *in vitro* formation of silica and other inorganic materials from aqueous precursor solutions under mild conditions. Here we present a summary of current insight into the mechanism of silica formation by diatom biomolecules and provide examples of synthetic (bio)polymers for the formation of silica and titania materials with complex structures.

## Introduction

Nature provides impressive examples of organisms that produce intricate inorganic structures (biominerals). For example, certain bacteria produce magnetic nanocrystals, many mollusks build calcium carbonate shells, and mammals form bone and teeth.<sup>1</sup> A remarkable characteristic of biomineral formation (biomineralization) is precise 3D control of complex mineral structures over several orders of magnitude in length scale (from a few nanometers up to millimeters or more). This is achieved by the action of highly organized assemblies of cellular macromolecules enabling mineral formation to proceed under physiological reaction con-

ditions.<sup>2,3</sup> Hence, understanding the molecular mechanisms of biomineralization may potentially provide novel routes for synthesizing complex inorganic materials under mild conditions. The present review is focused on materials syntheses inspired by diatoms, a fascinating group of biomineral-forming organisms that have been receiving increased attention from the materials science community.<sup>4-7</sup> Diatoms are single-celled algae that produce cell walls (frustules) of amorphous, hydrated silica with intricate, species-specific, 3D morphologies (Figure 1). The nano- to microstructured, highly symmetric, porous architectures of diatom frus-

tules have amazed natural scientists for more than two centuries.<sup>8</sup> However, only during the past decade have initial insights into the molecular mechanisms of diatom silica nanofabrication been gained. Here we describe how understanding of the molecular mechanism of silica formation by diatom biomolecules has been utilized to develop novel methods for synthesizing oxide/organic hybrid structures.

## Silica Formation *In Vitro* Using Biomolecules from Diatoms

Diatom biosilica is an organic-inorganic hybrid material containing biomolecules tightly attached/encapsulated within amorphous hydrated silica.<sup>9-12</sup> Over the past decade, three groups of biosilica-associated biomolecules (silaffins, silacidins, long-chain polyamines) have been identified that exhibit the ability to drastically influence the kinetics and structure of silica formation *in vitro*. Silaffins and silacidins are peptides or proteins that carry numerous phosphate residues attached to the amino acids serine and threonine.<sup>13-16</sup> Long-chain polyamines (LCPA) are non-protein components mainly composed of linear oligo-propyleneimine chains. Similar oligo-propyleneimine chains are attached to specific lysine residues in silaffins.<sup>17-20</sup>

Silaffin natSil1A (a natural mixture of the peptides natSil1A<sub>1</sub> and natSil1A<sub>2</sub>) was the first diatom component shown to accelerate silica formation from silicic acid solutions *in vitro*. The silica-forming activity of natSil1A is dependent on the presence of both phosphate-ester groups and oligo-propyleneimine chains.<sup>13,17</sup> This is consistent with the observation that silica formation by LCPA requires the presence of inorganic phosphate or other polyvalent anions.<sup>21</sup> Silica formed by natSil1A or LCPA-phosphate mixtures consisted of spherical particles in the size range from ~100–1000 nm. The mechanisms of silica formation in the natSil1A and the LCPA/phosphate systems appear to be very similar. In both cases, electrostatic interactions between polyamine chains and phosphate groups lead to the formation of supramolecular aggregates.<sup>13,21</sup> These aggregates appear to be responsible for accelerating the condensation of oligo-silicic acid molecules.<sup>21</sup> Only the polyamine moieties, but not the phosphate groups, are directly involved in catalysis of silicic acid polycondensation (see the next section). The natSil1A and LCPA/phosphate aggregates may directly act as templates for the formation of spherical silica particles. The size of the aggregates and the amount of oligo-silicic

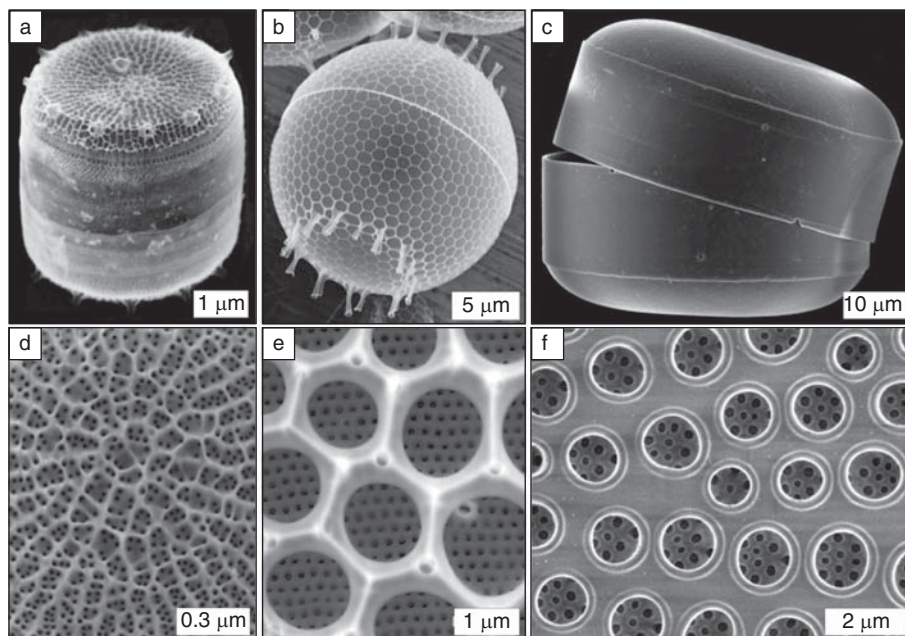


Figure 1. Scanning electron microscopy images of silica produced by living diatoms. (a, d) *Thalassiosira pseudonana*, (b, e) *Stephanopyxis turris*, and (c, f) *Coscinodiscus granii*.

acid that they bind may determine the final size of the silica spheres. This assumption is supported by studies with the synthetic LCPA-mimic polyallylamine.<sup>22</sup>

The formation of complex (non-spherical) silica structures *in vitro* using diatom biomolecules has been achieved by combining natSil1A or LCPA with so-called regulatory silaffins (natSil2, tpSil1/2H, tpSil3). In addition to being highly phosphorylated and containing polyamine-modified lysines, regulatory silaffins also carry sulfated carbohydrate residues. Regulatory silaffins lack inherent silica precipitation activity, which is due to the sulfated carbohydrate moieties that auto-inhibit the silica formation activity of the combined polyamine and phosphate groups.<sup>14</sup> However, when sufficient amounts of LCPA or natSil1A are added, supramolecular assemblies, termed the silaffin matrix, are spontaneously formed that exhibit silica precipitation activity.<sup>14,15</sup> The relative concentrations of LCPA/natSil1A and regulatory silaffins and the type of regulatory silaffin employed determine the amount and morphology of the silica. The complex silica structures formed by silaffin matrices include aggregates of pear-shaped particles, porous blocks, and plates (Figure 2b–2e).<sup>14,15</sup> Interestingly, the diameters of the pores in the porous blocks are in the range of 100–1000 nm, which is the char-

acteristic range of pore sizes in diatom silica frustules. The detailed mechanism(s) for formation of these complex silica structures is currently unknown. Dynamic light-scattering measurements have shown that the individual components of the silaffin matrix self-assemble into supramolecular entities.<sup>13,15,21</sup> It may be hypothesized that the silaffin matrix that forms porous blocks may consist of three different kinds of supramolecular particles: LCPA/natSil1A aggregates (10–20 nm diameter), aggregates of a regulatory silaffin (20–100 nm diameter), and mixed aggregates consisting of LCPA/natSil1A and a regulatory silaffin (~100–1000 nm diameter) (Figure 2f). The positively charged LCPA/natSil1A aggregates may act as electrostatic “glue” between the predominantly negatively charged regulatory silaffin and mixed aggregates, thus establishing a coherent 3D matrix of large aggregates that inhibit silica formation connected by areas that promote silica deposition. The addition of silicic acid to this system would yield porous blocks of silica (Figure 2f), which were observed in the experiments using mixtures of LCPA/natSil1A and a regulatory silaffin (Figure 2b–2e). It has been suggested that a similar mechanism may be responsible for silica formation in diatoms.<sup>11</sup> However, the existence of a silaffin matrix of such structure and properties *in vitro* or

*in vivo* has not yet been experimentally confirmed.

### Formation of Complex Silica and Titania Structures by Synthetic (Bio)Polymers

Insight into the mechanism of diatom silica biomineralization has stimulated research to identify readily available synthetic molecules that mimic the silica-forming abilities of silaffins and LCPA. Amino group-rich organic polymers (LCPA mimics) and lysine-, arginine-, or histidine-rich proteins or peptides (silaffin mimics) were found generally capable of precipitating silica from aqueous silicic acid solutions *in vitro*.<sup>23–29</sup> Furthermore, some of these molecules also deposited titania from aqueous solutions of water-stable Ti(IV) complexes (e.g., titanium(IV)-bis-lactato-bis-ammonium dihydroxide, TiBALDH).<sup>28,30,31</sup> A systematic study, using bacteriophage display-identified peptides, indicated that the titania-forming activity of such peptides increased with the density of positively charged amino acid residues.<sup>32</sup>

Silica-forming synthetic (bio)polymers are only active at pH values >6, while natural silaffin-LCPA aggregates are capable of forming silica down to pH 4.5.<sup>17,33,34</sup> This indicates that the silaffin-LCPA aggregates are particularly effective at promoting silicic acid polycondensation. The ammonium and amino groups of the oligo-propylamine chains of silaffins and LCPA are believed to act as acid-based catalysts for the condensation of silicic acid.<sup>34,35</sup> Figure 3a shows the proposed mechanism for catalysis of silicic acid condensation by oligo-propyleneimine-containing molecules, where R = H or CH<sub>3</sub>. Two consecutive repeat units within an oligo-propyleneimine chain are shown. Protonated and unprotonated amino groups may be present within the polyamine chain. The amino group and the ammonium group each bind a silicic acid molecule by a hydrogen-bonding interaction. Step 1: The amino group becomes protonated by taking over a proton from the silanol group (–Si–OH) of the bound silicic acid molecule, while the ammonium group donates its proton to the silanol group of the second silicic acid molecule. These proton exchange reactions result in formation of a reactive silanolate ion (–Si–O<sup>–</sup>) and transform the hydroxyl group of the neighboring silicic acid molecule into an oxonium ion. Step 2: The silanolate group attacks the positively polarized silicon center of the neighboring protonated silicic acid molecule, resulting in formation of a siloxane bond (–Si–O–Si–) and elimination of a water molecule. Step 3:

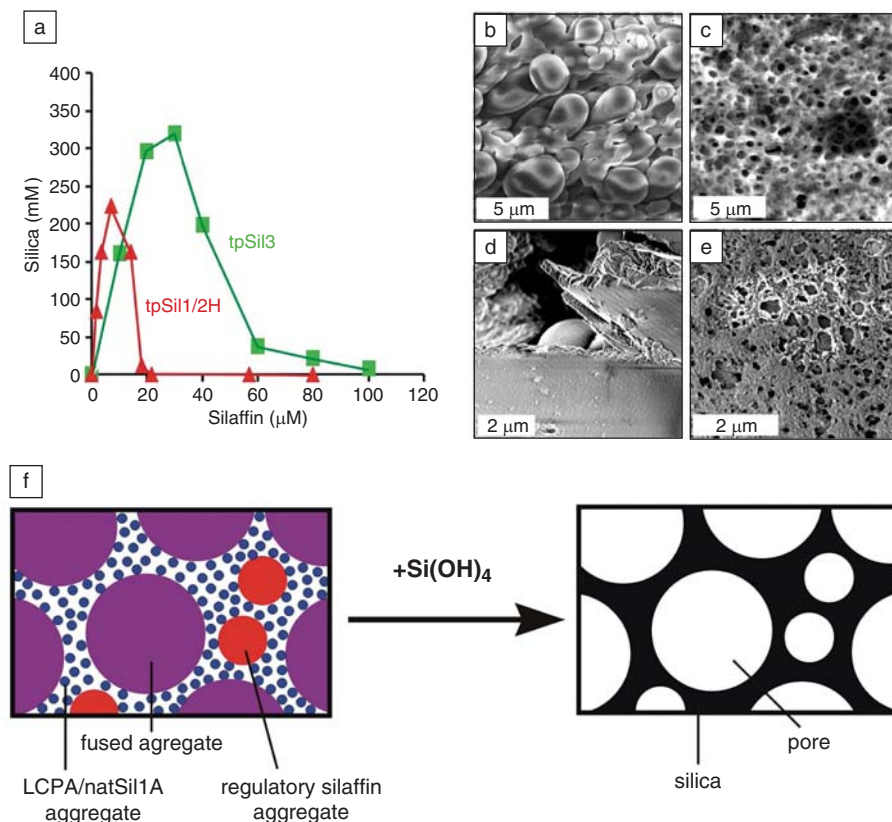


Figure 2. Control of silica morphogenesis by silaffin matrices. Silica formation experiments were performed in 100 mM silicic acid solutions at pH 5.5 with mixtures of silaffins and long-chain polyamines (LCPAs), as indicated.<sup>15</sup> (a) Amount of silica formed at a constant concentration of LCPAs and varying concentrations of silaffin tpSil3 (green) and tpSil1/2H (red). (b–e) Scanning electron microscopy images of silica formed by different silaffin matrices: (b) natSil2 + natSil1A, (c) natSil2 (2.5 lower concentration than in b) + natSil1A, (d) tpSil3 + LCPA, and (e) tpSil1/2H + LCPA. (f) Model for the formation of porous silica by silaffin matrices (see text for explanations).

The reaction products become displaced by two new silicic acid molecules, thus restarting the catalytic cycle. Additionally, the silicic acid molecules may become concentrated (relative to the bulk solution) within silaffin-LCPA aggregates via ionic and/or H-bond interactions with the numerous oligo-propyleneimine moieties, thus further increasing the reaction rate.

Under standard reaction conditions (i.e., unperturbed supersaturated silicic acid solutions around neutral pH at room temperature), most synthetic (bio)molecules induce the formation of spherical silica particles. Syntheses of spherical silica particles with well-controlled size ranges have already been established more than four decades ago via the Stöber process.<sup>36</sup> In the following, therefore, we will focus on examples for the use of synthetic (bio)polymers to form nonspherical silica and titania structures.

R5, a synthetic 19-amino-acid containing peptide derived from silaffin

natSil1A1, induces the formation of aggregates of spherical silica particles under standard reaction conditions. However, the addition of high concentrations of sucrose or the application of shear flow result in the formation of silica sheets or intertwined rope-like structures, respectively.<sup>17,37</sup> The shear flow-induced formation of rope-like structures indicates that the developing silica has a plasticity that can be affected by an applied hydrodynamic force field. The mechanism for the structural effect of sucrose is not understood.

Several authors have examined silicification of preformed scaffolds of R5-bearing fusion proteins. Pender et al.<sup>38</sup> fused the R5 peptide with the P1 peptide, which binds to single-wall carbon nanotubes. This enabled the formation of tubular silica and titania structures upon exposure to the appropriate precursor solutions. Foo et al.<sup>39</sup> synthesized a recombinant gene that encoded a fusion protein consisting of the

R5 peptide and a spider silk protein. The fusion protein was electrospun into fibers that, upon exposure to silicic acid, acquired a silica coating. Such fibrous protein-silica hybrid materials were considered for biomedical applications. Marner et al.<sup>40</sup> synthesized a fusion peptide that consisted of the R5 peptide fused to the C-terminus of the EAK<sub>1</sub> peptide (16 amino acids). The EAK<sub>1</sub> domain directed assembly of the fusion protein into a 3D hydrogel network, which formed a highly porous (<1 μm diameter pores) 3D silica structure upon exposure to silicic acid.

Tomczak and co-workers have demonstrated that synthetic poly-L-lysine (PLL) formed helical secondary structures in the presence of phosphate ions and silicic acid at pH 7.5.<sup>41</sup> The PLL helices self-assembled into hexagonal platelets that became silicified as the numerous amino groups of PLL catalyzed the formation of silica within these assemblies.

To investigate the mineral-forming properties of the polypeptide backbones of silaffins, Kröger et al.<sup>33</sup> synthesized recombinant DNA molecules encoding four silaffin domains: rSilC (17.6 kDa, pI = 11.8), rSilN (9.9 kDa, pI = 2.8), rSil1L (11.8 kDa, pI = 10.8), and rSil3 (22.1 kDa, pI = 9.4), where pI is the isoelectric point. After expression and purification of the recombinant proteins from *E. coli*, the three recombinant silaffins exhibiting pIs >7 were capable of silica formation from silicic acid, whereas only the two silaffins with the highest isoelectric points (rSilC, rSil1L) formed titania from an aqueous TiBALDH solution. The silica precipitates and the rSil1L-induced titania precipitate consisted of aggregates of spherical particles. The silica was amorphous, while the rSil1L-induced titania contained nanocrystals of anatase and monoclinic β-titania embedded within an amorphous matrix.<sup>33</sup>

The rSilC-induced titania consisted of highly crystalline particles of a complex structured rutile polymorph (Figure 3b–3f).<sup>33</sup> The overall shape of the rSilC-induced titania was spherical (Figure 3b), with each sphere composed of tightly packed columns of rutile crystals that were tapered in thickness (i.e., narrower at the sphere center and thicker at the surface). Numerous rectangular pores were present between the columns on the inside and on the sphere surface (Figure 3c–3d). The mechanism of formation of the columnar rutile structures is unknown. However, it was demonstrated that such rutile structures originated from an apparently unstructured, possibly nanocrystalline precipitate during dehydration with methanol and subsequent vacuum drying.<sup>33</sup> Unlike synthetic rutile



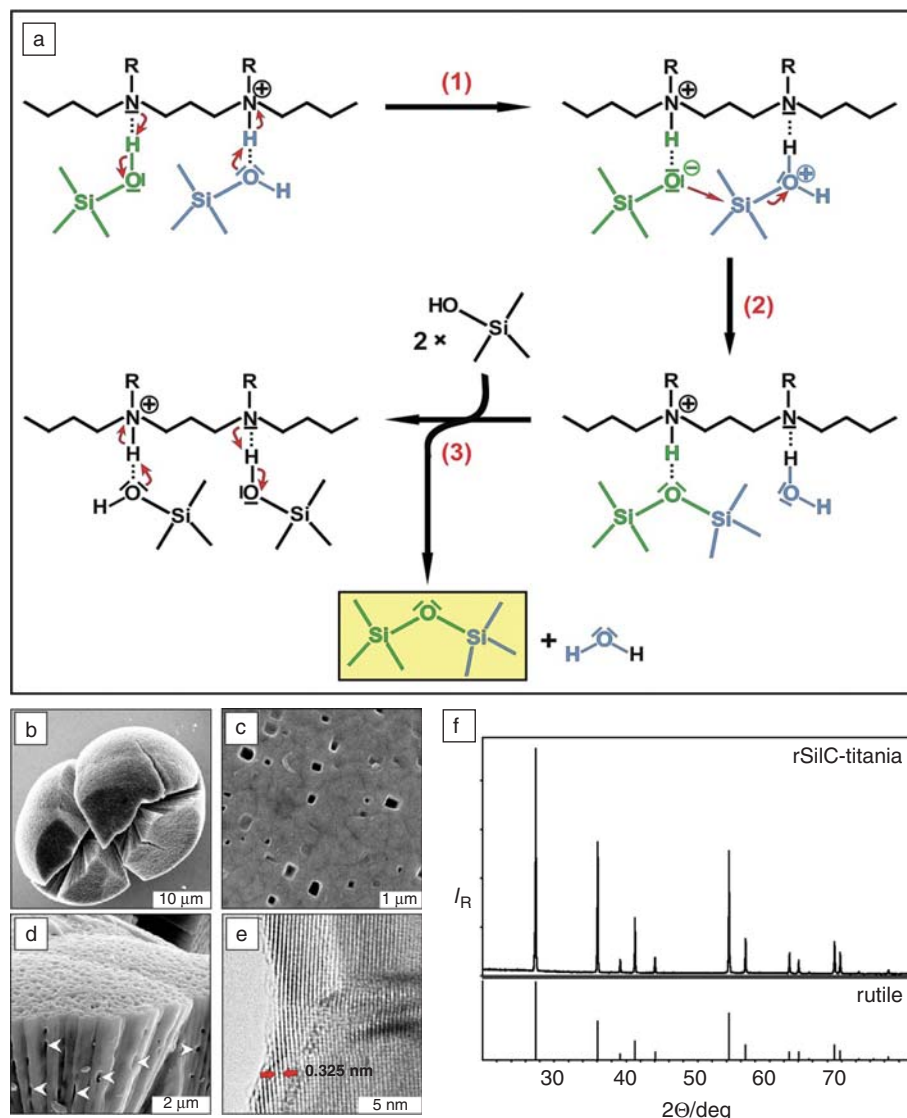


Figure 3. (a) Proposed mechanism for catalysis of silicic acid condensation by oligo-propyleneimine. R = H or CH<sub>3</sub>. (b–e) Rutile titania formation by recombinant silaffin rSilC from an aqueous solution of TiBALDH at pH 7.0 and ambient temperature. (b–d) Scanning electron microscopy images of rSilC-induced rutile microsphere: (b) overview of one particle; (c) detail from the outer surface of a particle containing multiple rectangular pores; (d) detail of the interior close to the external surface; the arrowheads indicate some of the pores inside the microspheres. (e) High-resolution transmission electron microscopy image of a microsphere fragment showing the (110) lattice fringes of rutile (arrows). (f) Powder x-ray diffraction analysis obtained from the rSilC-titania precipitate ( $I_R$  = relative intensity).

titania syntheses that require extremely acidic pH under hydrothermal conditions or firing at elevated temperature (typically at >800°C), rSilC-mediated synthesis of rutile was conducted at ambient temperature and neutral pH.

Organic polymers that mimic the polycationic character of LCPA also have been employed for the syntheses of silica structures. Sumper has used polyallylamine to synthesize porous silica structures<sup>42</sup> similar to those obtained using

silaffin-LCPA/natSil1A mixtures.<sup>14,15</sup> It was hypothesized that the silica sol nanoparticles became flocculated by free phosphate ions and arranged around the polyamine-phosphate microdroplets that served as templates for the polymerizing silica.<sup>42</sup> Indeed, the phosphate-dependent formation of nano- to micrometer-sized droplets of polyallylamine was subsequently demonstrated.<sup>22</sup> Lewis and co-workers<sup>43</sup> developed a direct ink-writing technique using a polyamine-rich ink to

produce solid 3D structures that exhibited diatom-like morphologies with ~1 μm-sized pores. Exposure of these scaffolds to silicic acid resulted in such sufficient silicification that the shape and integrity of the structure was preserved upon organic removal and partial sintering at 1000°C.<sup>44</sup>

## Concluding Remarks

The unmatched ability of living cells to direct the highly organized self-assembly of biomolecules is perhaps most strikingly displayed by the stunning variety of diatom frustule morphologies. Based on emerging insights into the molecular mechanisms of diatom silica formation, novel (bio)polymer-based approaches for the syntheses of complex mineral structures have been developed for silica and titania (as described here) and also for germania and titanium phosphate.<sup>28,31,45</sup> In parallel, results from studies on other biomineralization systems (most notably silica sponges and magnetite-forming bacteria) have been exploited for materials syntheses.<sup>46–48</sup> Initial successes in the chemical modification of diatom frustules through *in vivo* functionalization also have been achieved.<sup>49</sup> Despite these encouraging results, replicating the intricate 3D morphologies of natural biominerals *in vitro* by self-assembly of mineral-forming (bio)polymers has not yet been achieved. This demonstrates our incomplete knowledge of the components involved in biomineralization and gaps in fundamental understanding of the mechanisms of biomineralization. To be able to fully harness nature's ability for mineral formation both *in vivo* and *in vitro*, continued efforts in fundamental biomineralization research are required.

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